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The role and importance of the larvae of St. Mark's fly in the formation of a rendzina soil

INTRODUCTION

The soil formation process taking place on so-called "leithalimestone", under a mixed forest crop of *Quercus cerris, Q. pubescens, Q. petraea, Carpinus betulus,* etc. was studied near Sopron, Western Hungary, with particular regard to the role of *Bibio* larvae. Soil type: humid rendzina. Variety: mull-like rendzina. Figure 1 presents the chemical composition of a soil profile between 5-40 cm / 0-5 cm: L- and F-layers/. The $CaCO_3$ and humus contents are high (A), the exchangeable calcium dominates in the colloidal complex (B) and the conditions are favourable as regards the amount of easily available potassium (according to Nehring; C). The soil is very poor in clay minerals. Its micro-structure is mull-like moder.

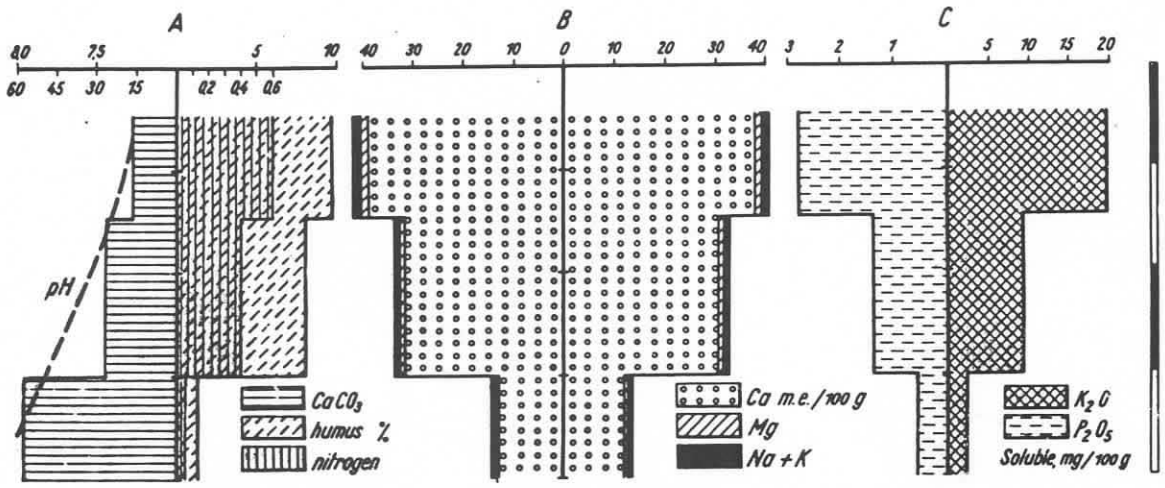

Fig. 1
The chemical composition of the investigated mull-like rendzina soil-profile.

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OCCURENCE AND FREQUENCY

The *Bibio* larvae, observed on the examined area, belonged to the species *B. marci* L. (*B. hortulanus* L. var. *marci* L.). Both larvae and bred imagines were determined, the former on the basis of the microscopical cuticular structures of abdominal- and thoracic segments. These organisms live on the border of the L- and F-layers, in populations (in the so-called "Fressgemeinschaften") usually consisting of 500-2000 individuals. The members of these populations always remain together. Populations consisting of more than 1500-2000 individuals cannot possibly originate from the egg-deposit of a single female. A typical case of the distribution of these larva-populations (3.9.1963) is shown in Figure 2.

On an area covering 72 m^2, 36 Diptera populations, belonging - with one exception: No. 16, Mycetophilidae (?) pupae - to the species *B. marci* L. were observed. In Figure 2 each of them is represented by the sketch of a larva.

Figure 3 contains data on biomass and the number of individuals of one part (Nos. 1-15 and 17-21) of the same larva-communities, at two different points in time (3.9.and 12.12.1963). With the winter approaching, the total weight of living larvae increases significantly although the number of individuals usually decreases. This latter fact may be chiefly attributed to predatory beetles and their larvae. One living *Bibio* larva weighed 5,6 $\pm$ 1,1 mg on 3.9.1963 and 62 $\pm$ 2,9 mg on 12.12.1963.

The circular diagram (Figure 4) represents the distribution of the percentage number of individuals and the weight distribution of the biomass of the macro- and megafaunas on 1 m^2 of the L- and F-layers, on the basis of data coming from 10 sampling areas /8.11.1960/. The absolute dominance of Diptera-larvae is obvious, and of them the *B. marci* larvae constituted more than 80 per cent of the number of individuals. Figure 5 shows the seasonal changes in the numbers of the different groups of the fauna of the L- and F-layers between 2.4. and 8.11.1960, along with the fluctuation of the water content of the several years old lower litter (A"00) and of the F-layer.

In Figure 5 curve "Diptera I" represents the total amount of the members of the Solvidae and Stratiomyidae families and curve "Diptera II" represents that of the Bibionidae, Tipulidae, Muscidae, Lycoriidae and Ceratopogonidae families. Since the total number of individuals of the latter four families remains always below 12 larvae/m^2 - apart from

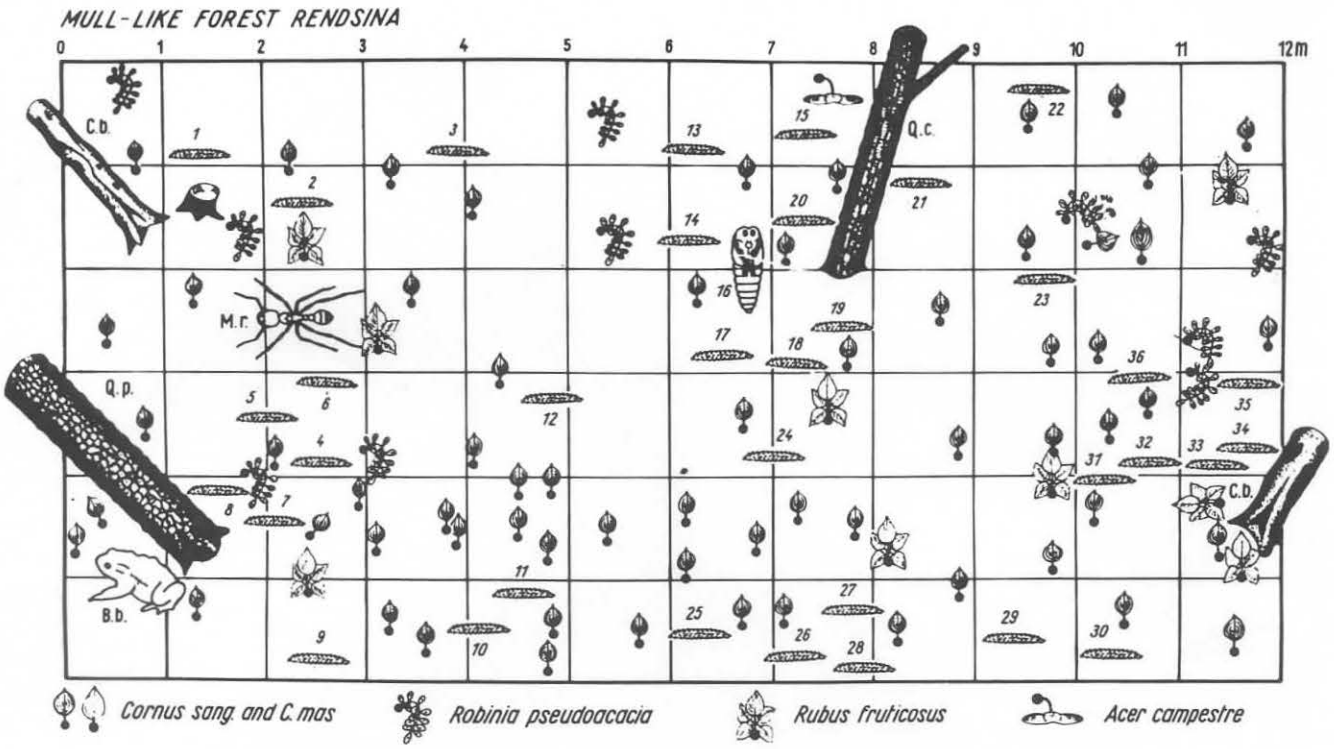

Fig. 2
The distribution of B. marci larva-populations on a mull-like rendzina "pedon". C.b. = Carpinus betulus; Q.c. = Quercus cerris; Q.p. = Q pubescens; M.r. = Myrmica ruginodis nest; B.b. = the entrance of the subsurface burrow of Bufo vulgaris. Larvae marked 1-15 and 17-36 each represent one Bibio-population. Pupa No 16: Mycetophilidae (?) pupa-colony.

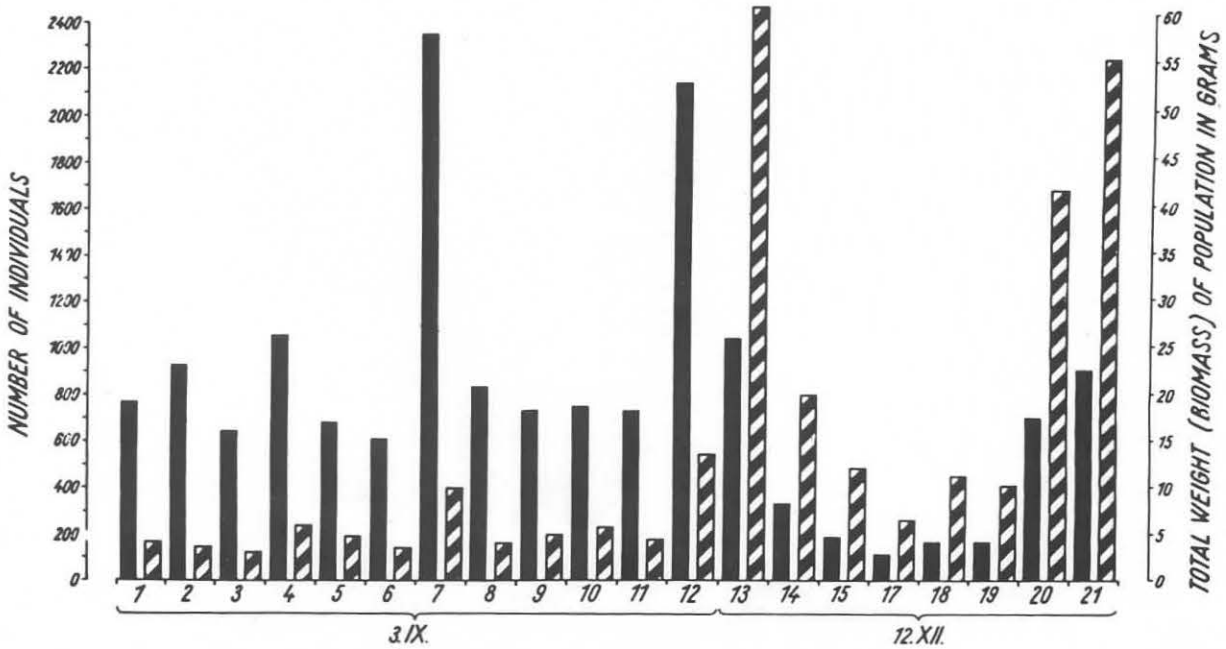

Fig. 3
The number of individuals and biomass of Bibio marci populations.

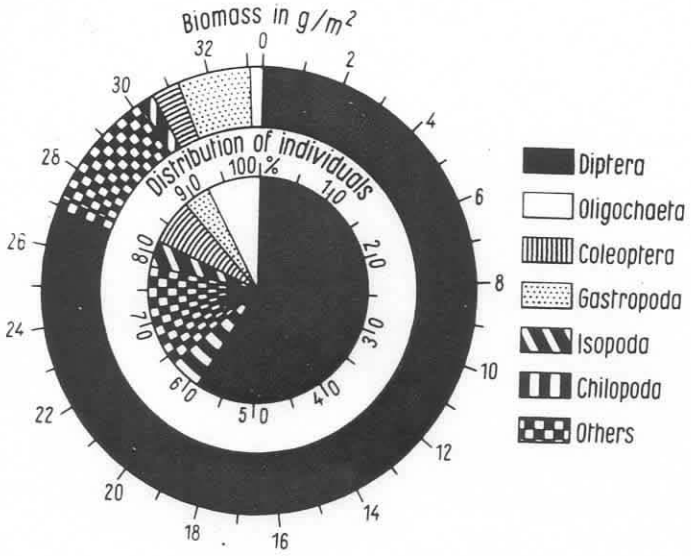

Fig. 4
Distribution of the biomass and the number of individuals in the macro-and megafaunas of the L- and F-layer.

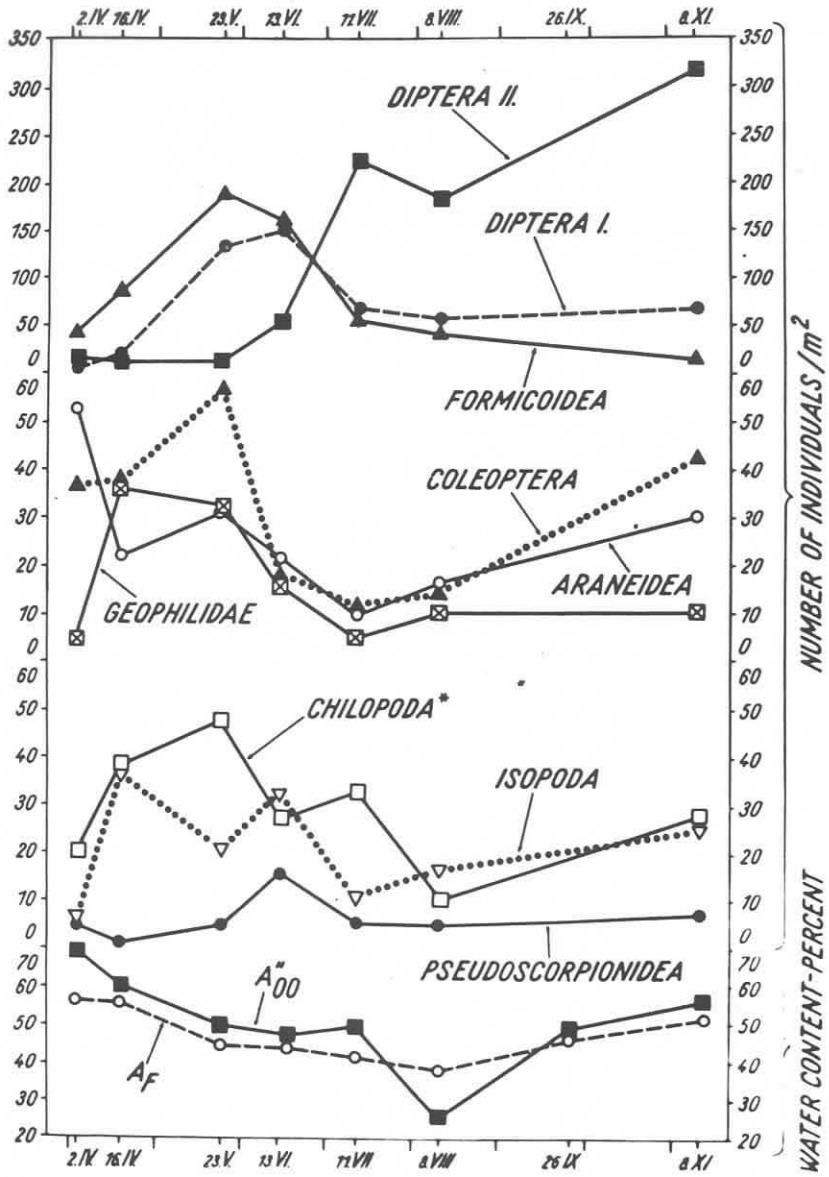

Fig. 5
Seasonal fluctuation of the number of individuals in the macro-and mega-faunas of the L- and F-layers.

the survey data obtained on June 13 - curve "Diptera II" actually indicates the number of individuals of the *B. marci* larvae from July 11. The form of this curve indicates the one-generation appearance of the species: the imago in April - May, the main activity period of the larvae at leaf fall and overwintering on the soil surface. The published data make it evident that the *B. marci* larvae are the absolutely dominant members of the macro- and megafauna, controlling the transformation of organic matter and the degradation of plant remains in the L- and F-layers, during the most important autumn period.

NUTRITION

According both to observations carried out in the natural environment and examinations of the intestinal content and the faecal pellets, the larvae are (phyto-) saprophagous. Nevertheless they consume mainly the fallen leaves of hornbeam and oak that may be found near the larva-populations in large quantities on the soil surface, perforated and eaten away to the woody leaf veins (Figure 6 upper left picture).

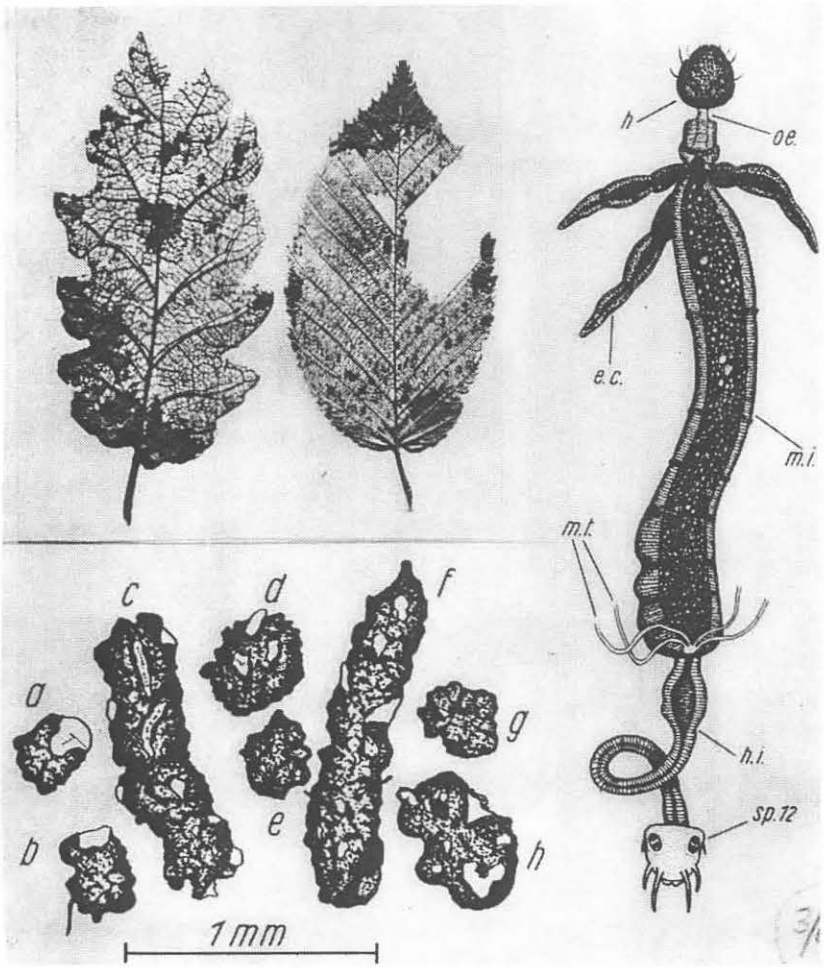

Fig. 6
Oak- and hornbeam leaves eaten by Bibio-larvae. Bibio excrement particles. Sketch of the intestinal canal: h = head; oe. = oesophagus; e.c. = enteric coeca; m.i. = mid intestine; m.t. = Malpighian tubes; h.i. = hind intestine; sp.12 = spiracle 12 .

They also take up, that is, pass through their digestive canal, other plant and animal residues from the F-layer and large amounts of mineral particles from the upper layer of the A_H-horizon as well as the humified organic matter of the soil. In the fresh excrement the following materials were identified (Fig.7):

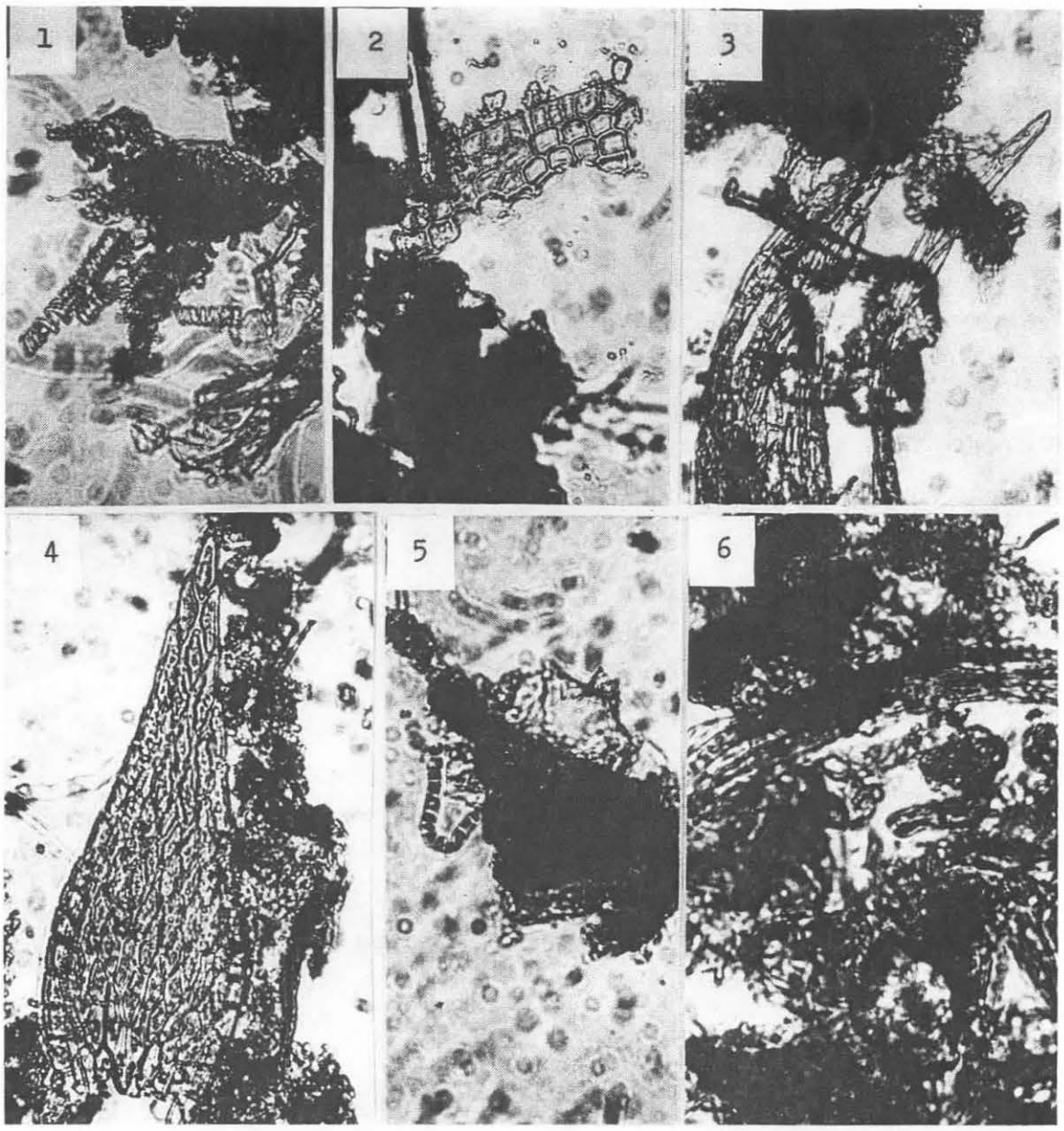

Fig. 7
Organic structures in the fresh excrement of Bibio-larvae.

plant fibres, pieces of cell-structured plant tissues, mineral particles (mainly calcite), various spiral-like structures, green alga-filaments, structureless (cloud-like under microscope) organic matter formations, cysts of Protozoa (but not active forms!), eggs, etc. The excrements

are blackish, dark coloured, their surface is uneven, their width is 0,2-0,3 mm and their length is sometimes more than 1 mm (Fig 6, lower left picture). In the alimentary canal of the animals (in Fig. 6, the intestinal canal is shown in a stretched position) the coarsely dispersed materials pass straight through the mid-intestine. It can be clearly seen in the Figure that the dark intestinal content is full of light coloured calcite particles. In the three large enteric coecas, a portion of the finely dispersed materials may be found. It is probable, that the microbial communities of these latter play an important role in the continuous inoculation of nutriments. The litter is transformed into excrement mass most intensely from September to November but the larvae take nourishment in winter too.

THE ROLE OF THE LARVAE IN SOIL FORMATION

Under individual larva-populations large amounts of excrement accumulate, displaying a characteristic picture in thin soil sections (Figure 8).

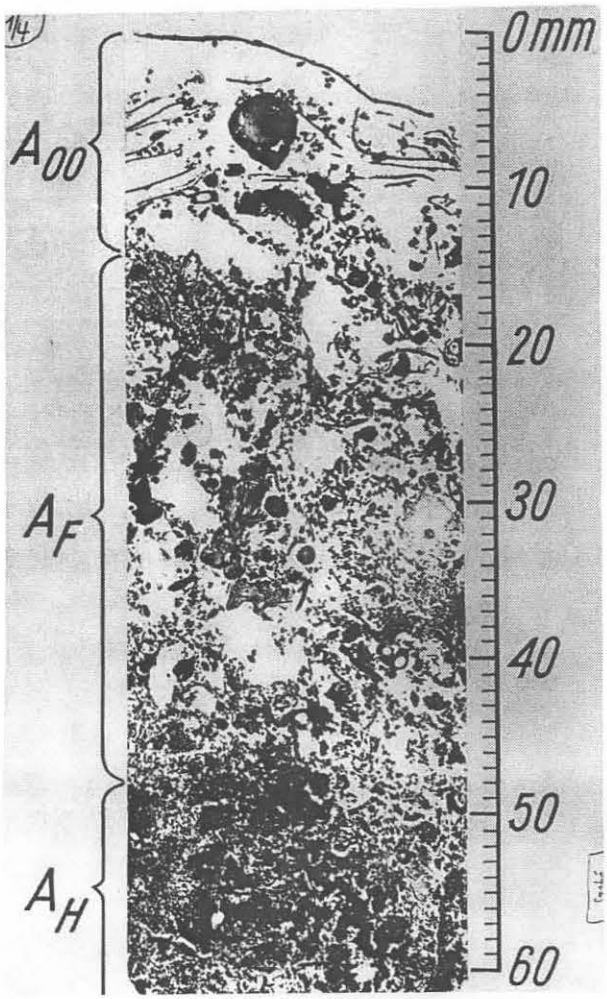

Fig. 8
Thin soil section with the cross section of a coprogenic aggregate mass accumulating under a Bibio larva-population. The characteristic little black Bibio-excrements are frequent on the leaves (L-layer). They have accumulated in a large mass in the upper left part of the F layer, and it is easily observable that Bibio excrements (remaining from the earlier years) are important constituents of the soil matter of the A_H-horizon too.

The cross-section of the excrement particles is of mosaic-like structure (Figure 9) owing to the heterogenous distribution of organic matter and mineral particles.

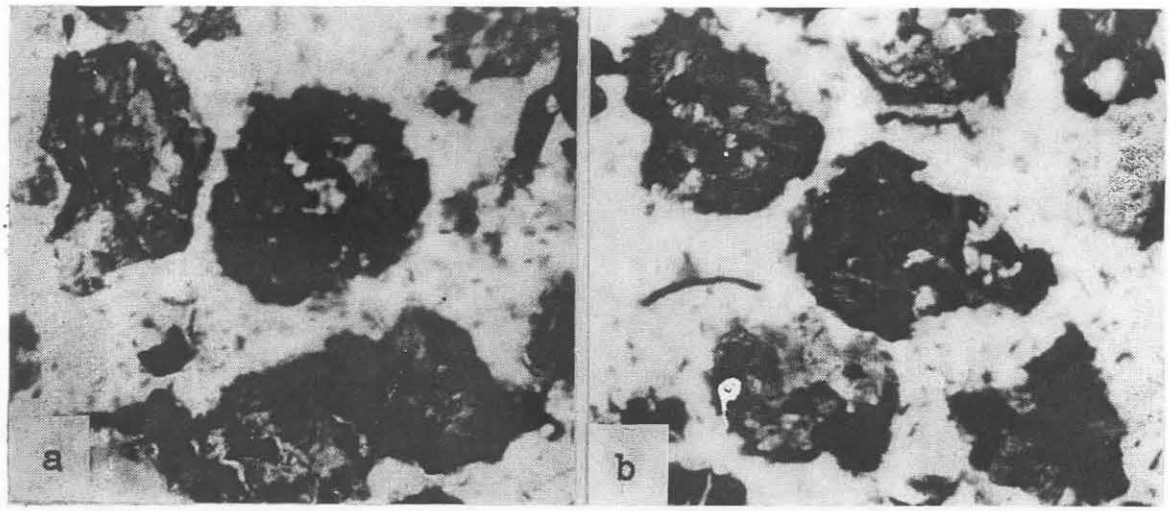

Fig. 9
a and b, accumulated excrements of Bibio larvae. Thin soil section prepared with "Polikon ES" (unsaturated polyester cast-resin).

The excrement of the larvae effervesces on treatment with dilute hydrochloric acid, it is rich in calcite and these organisms do not show sensitivity to lime.
If we estimate the quantity of larvae at 200 per m^2 at least, then according to both our observations and the data given by d'Aguilar and Bessard (1963), these organisms annually produce 100 g of excrement, calculated, in dry matter, for an activity period of 100 days. The total amount of fallen litter - including the remains of grasses (Gramineae) - is annually 300 g per m^2 in dry matter on the average. This litter constitutes 40-50 per cent of the animals' feeding (more exactly consumed) materials. In an activity period 200 larvae per m^2 consume approximately 15 per cent of the litter. Interesting calculations may be made if we compare these data with the weight distribution of the A_H-horizon. 1 m^2 of a 30 cm deep A_H-horizon represents 314 kg of material on the average. From this, stone and gravel larger than 2 mm in diameter weigh 67 kg, the living roots 2,2 kg, the dead roots of ligneous plants 0,76 kg, the dead roots of herbaceous plants 1,3 kg and finally soil containing particles smaller than 2 mm in diameter weighs 243 kg. At places, where the frequency of these organisms is 200 larvae per m^2, the total amount of excrement produced in one year corresponds to 0,04 per cent of the soil matter.

The thickness of the A_H-horizon is often only 10 cm. Calculation with the above data, in this case the excrement produced by a population of 200 larvae per m^2 in 100 days would be 0,12 per cent of the soil matter. An even better insight into the consequence of the activity of the larvae may be obtained if we compare the data of the chemical analysis of the excrement mass (Table 1), accumulated under the larva-populations and separated under a stereo-microscope, with the corresponding data of the upper litter (A'_{oo}), of the several years old, partly decomposed lower litter (A''_{oo}) of the F-layer and finally with those of the soil (A_H 5 cm). According to these data, the C : N ratio becomes gradually narrowed: A'_{oo} 30,1 > A''_{oo} 18,2> excr. 12,6> A_H 7,8. Only the F-layer, accumulated partly from organic residues which are poor in nitrogen and decompose with difficulty, shows deviation from this tendency. The high ash content and the low carbon content of the excrement show that the larvae consumed not only organic matter but mineral particles - in considerable quantities too. Here two facts should be noted. First, since the nutrition of the animals is essentially different under natural circumstances and during nutrition experiments, the chemical analysis of excrements collected under living larva-populations on the original place of their activity may supply more true information concerning the soil formation process. Secondly, it was not the fresh excrement but the ex-

Table 1

	Carbon %	Nitrogen %		C/N Ratio	Ash %
		Total	Soluble		
A'_{00} Upper litter: leaves of hornbeam and oak	43,04	1,43	0,21	30,1	10,14
A''_{00} Lower, partly decomposed litter	31,19	1,71	0,21	18,2	23,16
A_F Fermentation F-layer	22,79	1,10	0,08	20,7	50,05
Accumulated faecal pellets	11,33	0,89	0,096	12,6	65,24
A_H (5 cm) Humus-layer	3,59	0,461	0,06	7,8	81,58

crement mass accumulated for several weeks that we analysed because the decisive chemical change takes place under the influence of those microorganisms which - although they became selected and activated in the milieu of the intestine - perform their prolonged activities in the excreted faeces, (Szabó and Marton 1966).

THE INFLUENCE OF LARVAE ON MICROBIAL DYNAMICS

In the course of their nutrition, the larvae infect their intestinal canals with numerous species of soil microflora taken up with the organic matter of the soil. According to our examinations, a characteristic microflora is selected in the intestinal canal already at an early stage of development. This shows a rather restricted (poor in species) aspect if compared to soil microflora. The specificity of intestinal microflora is only relative, because - depending on the environmental circumstances - not always the same species are consistently selected (Szabó and Marton 1966).

Figure 10 presents the schematic picture of the intestine- and excrement microfloras of a larva-population as well as that of the microbial communities of their nutriment sources. Although the figure illustrates only the most important species and the ones - even if they occur only infrequently - which are characteristic, the complexity of analysing such microflora changes can be realized.

Figure 11 presents electron microscope photographs of two characteristic microorganisms of the intestine and excrement. In the course of our experiments we endeavoured to obtain information concerning the role of microflora, too..

Figure 12 shows the free amino acid constituents of the L- (A^{I}_{oo}, A^{II}_{oo}) and F-layers, of the accumulated excrements and of the soil (A_H 5 cm). Here we also present the distribution of free amino acids traced from liquid synthetic cultures of certain strains of streptomycete species that are common both in the *Bibio* intestine and in soil. It can be seen, that the litter fallen in autumn has the highest free amino acid content, the lower litter and the F-layer being in the second and third places respectively. We succeeded in tracing 4 spots each in the excrements and in the soil. The presence of α-alanine, glutamic acid and aspartic acid were characteristic in both substrata. These are the so-called "main amino acids"

directly connected with the Krebs-cycle. They may be detected in every streptomycete culture belonging to the dominant species of excrement and of soil. It seems to be obvious that these organisms are responsible for the free amino acid composition of both media.

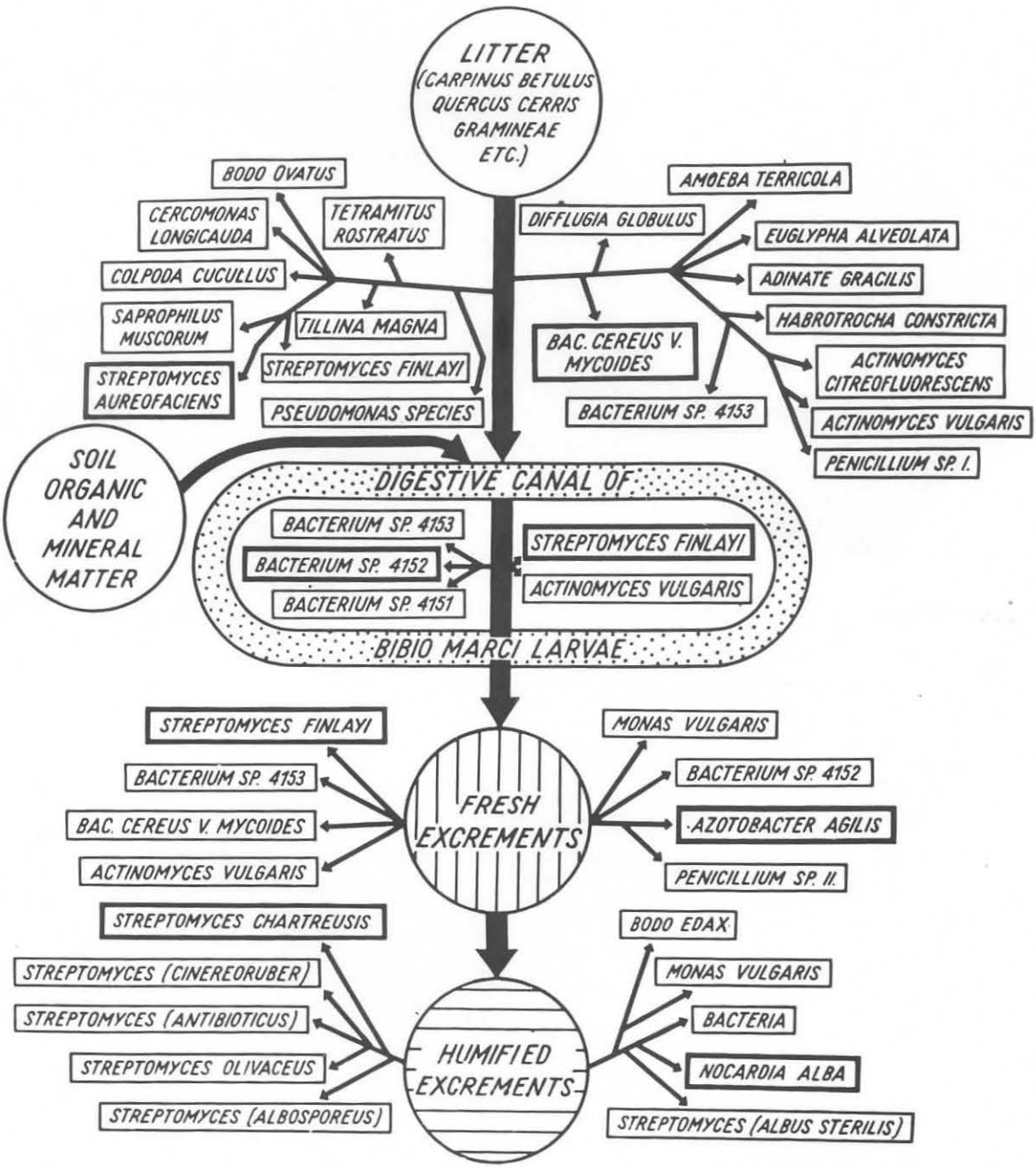

Fig. 10
The alteration of the composition of microbial communities under the influence of Bibio larvae in one of the cases studied. The arrows indicate the direction of the flow of organic matter and energy. (Actinomyces = Streptomyces; the thick frame means very common (dominant-) occurence; the systematic determination of the organisms designated with numbers is under way.)

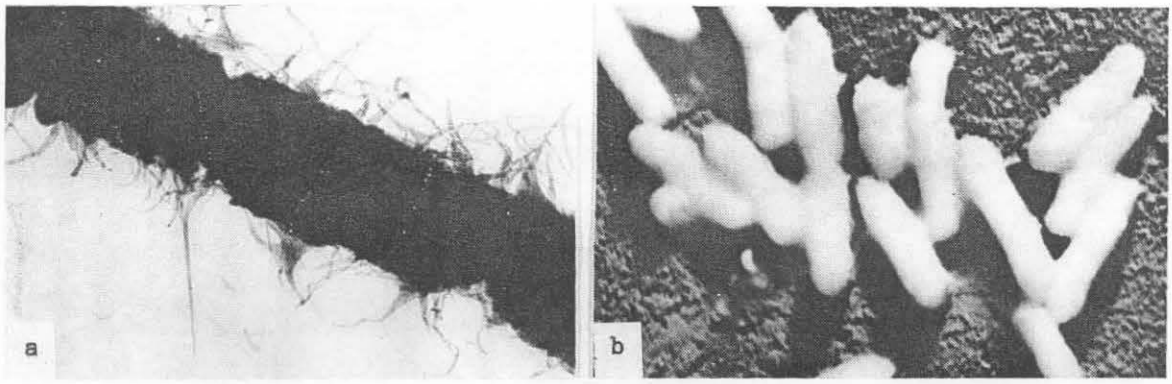

Fig. 11
A chain of hairy spores (a) of a strain of Str. finlayi (x 8,000) and the cells (b) of Bacterium sp. 4152 (x 4,400) isolated as dominant types from the intestinal microflora of different individuals of a Bibio marci larva-population.

Table 2 displays the nitrogen source utilization (on the synthetic media of Pridham and Gottlieb) of *Streptomyces finlayi*, which is dominant in the intestine. It can be seen, that this organism is capable of utilizing most amino acids - as single N-source in the medium - and it is quite probable that the population of this species is mainly responsible for the vanishing of the autumn litter's free amino acid content.
A detailed account of our work will be published elsewhere.

Table 2
Utilization of nitrogen compounds by Streptomyces finlayi

NH_4Cl	± − +	DL-Methionine	+
$(NH_4)_2CO_3$	+ + +	L-Arginine	+ + − + + +
NH_4NO_3	+ − + +	DL-Lysine	+ +
$(NH_4)_2SO_4$	+ − + +	DL-Aspartic acid	+ + − + + +
Urea	+ + +	L-Asparagine	+ +
Glycine	+ +	L-Glutamic acid	+ +
DL-Alanine	+ +	L-Tyrosine	+
DL-Valine	+ + +	DL-Tryptophan	+ + − + + +
DL-Norvaline	+ − + +	L-Histidine	+ +
DL-Serine	+ − + +	Nucleic acid	± − +
DL-Threonine	+ + − + + +	Peptone	+ − + +
Cysteine	+ +	Tryptone	+ +
L-Cystine	±	None	0

0 = no growth ; ± = very slight growth ; + = poor growth;
+ + = fair growth; + + + = good growth.

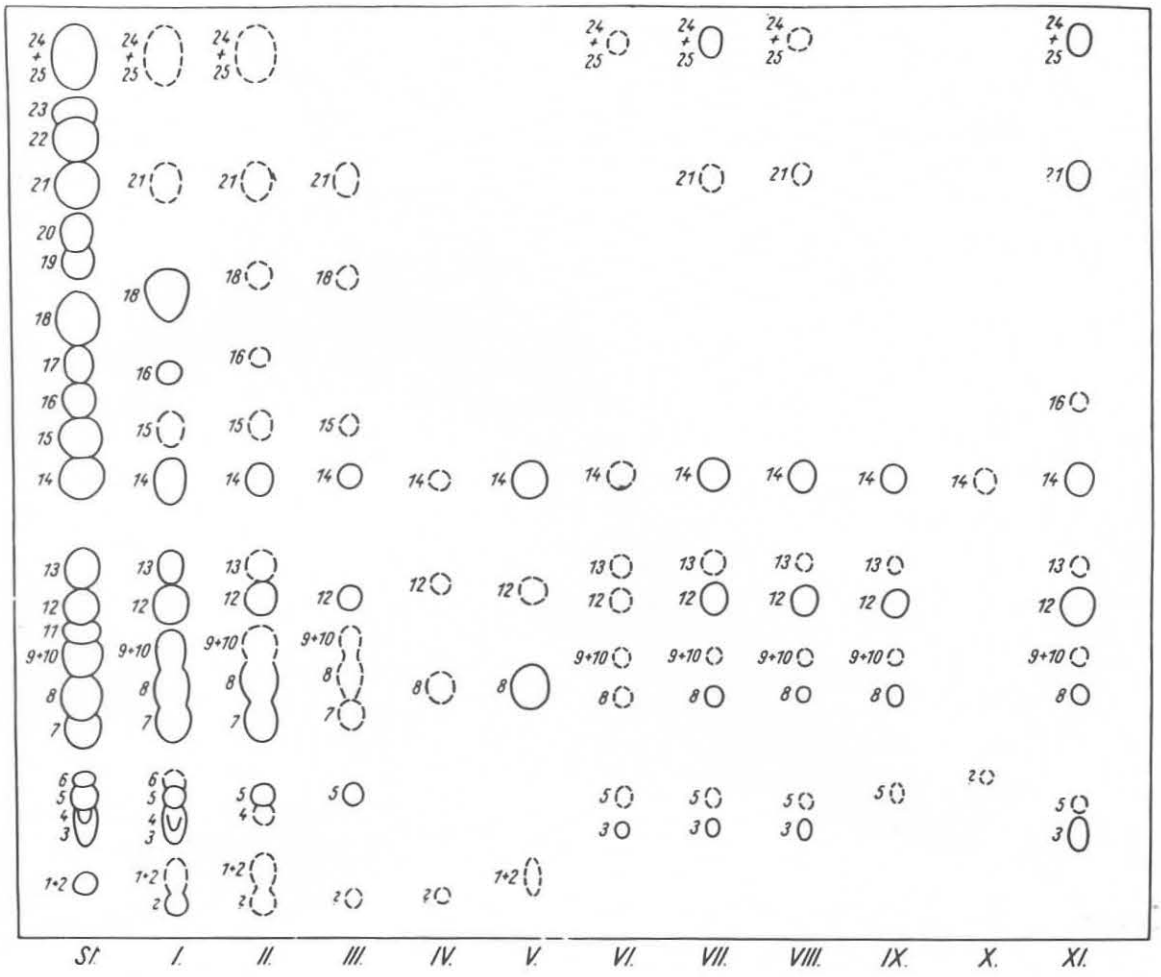

Fig. 12

Comparative paper-chromatographic analysis of free amino acids. The results are presented in schematic representation. Dotted spots were very faint. Solvent: butanol: acetic acid: water (4: 1:5). Filter paper: Schleicher-Schüll No 2043a.

St. = Standard; I. = A'_{00}; II. = A''_{00}; III. = A_F; IV. = Faecal pellets; V. = A_H/5cm/; VI. = Streptomyces vulgaris 4155; VII. = Str. finlayi 4161; VIII. = Str. finlayi 4162; IX. = Str. finlayi 4163; X. = Str. sp. 4165; XI. = Str. finlayi 1869 / holotyp /; 1-2 = Cysteine + Cystine; 3 = Lysine; 4 = Histidine; 5 = Arginine; 6 = Asparagine; 7 = Glutamine; 8 = Aspartic acid; 9 + 10 = Glycine + Serine; 11 = Oxyproline; 12 = Glutamic acid; 13 = Threonine; 14 = α - Alanine; 15 = β - Alanine; 16 = Proline; 17 = Tyrosine; 18 = α-Amino butyric acid; 19 = Tryptophan; 20 = Methionine; 21 = Valine; 22 = Norvaline; 23 = Phenylalanine; 24 + 25 = Leucine + i-Leucine.

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DISCUSSION

M.S. GHILAROV: In der Kursker Region fressen die *Bibio*-Larven nicht die ganze Streumasse, sondern nur kleine Stückchen von Ästen und Zweigen, so daß man sie nicht als saprophag, sondern eigentlich als sapro-xylophag bezeichnen müßte. Vielleicht ist es so, daß dort die Streu rasch austrocknet, während innerhalb der Ästchen das Substrat längere Zeit feucht bleibt.

I. SZABO: Die Beobachtungen von Herrn Professor Ghilarov weisen darauf hin, was übrigens auch wir feststellen konnten, daß die Zusammensetzung der Nahrung der *Bibio*-Larven gemäß den Milieu-Verhältnissen bedeutende Unterschiede zeigt. Dementsprechend ist die Rolle und die Bedeutung dieser Organismen im biozönotischen Stoffkreislauf sowie unter dem Gesichtspunkte der landwirtschaftlichen Produktion keineswegs immer die gleiche.

D. MOSSAKOWSKI: Berücksichtigen Sie bei der Berechnung der Ähnlichkeitskoeffizienten Korrelationen der Merkmale oder werden die Unterschiede summiert?

I. SZABO: Die systematische Determination und den Vergleich der aus dem Darminhalt und den Exkrementen isolierten Mikroorganismen haben wir auf Grund von 76 - 84 Eigenschaften durchgeführt. Der Ähnlichkeits-Index (S) der einzelnen Stämmepaare (sowie die "Gruppenanalyse") wurde mit einem elektronischem Computer gerechnet und zwar nach der Formel: $[S = 100\ (a+d)\ ] / (a+b+c+d)$, wo a bzw. d die Summe der gemeinsamen positiven bzw. negativen Merkmale bedeuten, b und c bedeuten die Summe der Merkmale, die sich als + und − bzw. − und + bei den beiden verglichenen Stämmen zeigten.

G. ZACHARIAE: In der Tabelle 1 ist der Aschegehalt der Kotballen wesentlich höher als in der Streu. Das beruht zweifellos auf mitgefressenen Mineralkörnern. Vermischung mit Mineralsubstanz fördert erfahrungsgemäß die Zersetzung, und jeder Beitrag von Tieren dazu gilt als humusbiologisch wertvoll. Können Sie entscheiden, ob die Larven regelmäßig nach Mineralkörnern suchen oder nur zufällig die an den Blättern haftenden aufnehmen? Liegen an Ihrem Standort die Blattreste in der F-Schicht dem Mineralboden auf, so daß eine Verschmutzung eintreten muß, oder sind sie durch stärkere Schichtdicken davon getrennt? Gelingt eine Zucht der Larven ohne Beigabe von Mineralkörnern?

I. SZABO: Der Darminhalt der Tiere, die auf ihrem natürlichen Standort gesammelt wurden, enthält immer - ohne Ausnahme - große Mengen von Mineralkörnern, auch dort, wo (oberste Förna) das Milieu sehr arm an Mineralteilchen war. Nach unseren Beobachtungen findet die Aufnahme der Mineralsubstanzen durch die *Bibio*-Larven regelmäßig statt und ist keineswegs Zufall. Doch ist die lang andauernde Zucht der Larven unter Laboratoriumsverhältnissen auch bei Vorhandensein von Mineralkörnchen problematisch, da diese Tiere gegenüber einer Reihe von Umweltfaktoren sehr empfindlich sind. Es ist bemerkenswert, daß von den im Dezember gesammelten Larven noch viele bis zum Frühling am Leben geblieben sind, wenn wir sie im Kühlschrank zwischen 2 - 4°C gehalten haben.